

# A Calvin bestiary

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## Abstract

This paper compares a number of mathematical models for the Calvin cycle of photosynthesis and presents theorems on the existence and stability of steady states of these models. Results on five-variable models in the literature are surveyed. Next a number of larger models related to one introduced by Pettersson and Ryde-Pettersson are discussed. The mathematical nature of this model is clarified, showing that it is naturally defined as a system of differential-algebraic equations. It is proved that there are choices of parameters for which this model admits more than one positive steady state. This is done by analysing the limit where the storage of sugars from the cycle as starch is shut down. There is also a discussion of the minimal models for the cycle due to Hahn.

## 1 Introduction

The Calvin cycle is a part of photosynthesis and there are many mathematical models for this biochemical system in the literature. Reviews of these can be found in [2], [10] and [3]. The aim of this paper is to survey what is known about the dynamics of these models with a focus on what has been proved rigorously. It should be pointed out right away that the rigorous results constitute a small island in an ocean of simulations and heuristics. To start with it is necessary to fix the boundary of the area to be covered. The models treated are all deterministic, continuous time evolution equations without delays and spatial variations are neglected. Thus mathematically we are dealing with systems of ordinary differential equations (ODE) or differential-algebraic equations (DAE). The unknowns are concentrations of chemical substances depending on time.

Photosynthesis is a process of central importance in biology and, as a consequence, in our daily lives. It consists of two major parts. In the first of these (the light reactions) energy is captured from sunlight and molecular oxygen is produced. In the second (the dark reactions) carbon dioxide from the air is used to make carbohydrates. For reasons to be described later the second part

is also called the Calvin cycle. The models which are the subject of what follows relate to the Calvin cycle and all describe ordinary chemical reactions in solution together with simple sources, sinks and transport processes between cellular compartments which fit into the same mathematical framework. The light reactions involve electrochemistry on a membrane, a type of process whose modelling will not be considered here. A comprehensive introduction to the biochemistry of photosynthesis can be found in [9].

If we are describing one biological system here, why should there be many mathematical models for it? This is a consequence of some general features of the modelling of biochemical systems which will now be listed. The first is that a biochemical system like the Calvin cycle is in reality coupled to many other chemical processes (the light reactions, sucrose production etc.) and so we have to make a choice of the set of chemical species whose concentrations are included as unknowns in the ODE system. The hope is that these concentrations have only a small effect on the concentrations of the other species with which the chosen ones interact. The concentrations of these other species are taken to be constant and we refer to them as external species while the species whose concentrations are the unknowns in the ODE system are referred to as internal species. A possible justification for this procedure is that if the concentration of an external species is very high it will remain approximately constant even if some amount of the substance concerned is being produced or consumed by some of the other reactions. There is also a choice of which reactions are considered to be taking place at an appreciable rate. Usually the stoichiometry of the reactions is known but the same cannot be said of the reaction rates. There further assumptions have to be made. Summing up, different mathematical models arise through different choices of the species and reactions included and the reaction kinetics. Furthermore it may happen that models are replaced by smaller ones using limits involving time scale separation or elimination of intermediate species in some reactions.

After these preliminary considerations we may look at what a standard textbook on cell biology [1] tells us about the Calvin cycle. The essential features of this process were worked out by Melvin Calvin and his collaborators (earning Calvin the Nobel prize for chemistry in 1961). Often the situation of carbon dioxide and light saturation is considered. Calvin's experiments were done under these circumstances and they are often assumed to hold when doing modelling. This means on the one hand that carbon dioxide is so plentiful that it can be considered as an external species. On the other hand the substances ATP and NADPH which are supplied by the light reactions are assumed to be plentiful. Thus  $\text{CO}_2$ , ATP and NADPH are taken as external species. The same is true of ADP and NADP which are produced from ATP and NADPH in certain reactions. Inorganic phosphate  $\text{P}_i$  is often also treated as an external species. All the reactions are catalysed by enzymes but these are usually treated as external species. The substances which remain in the description in [1], and which will be internal species, are ribulose 1,5-bisphosphate (RuBP), 3-phosphoglycerate (PGA), 1,3-bisphosphoglycerate (DPGA), glyceraldehyde 3-phosphate (GAP) and ribulose 5-phosphate (Ru5P). The simplest assumption is that each of these

substances reacts to give the next with a final reaction taking us back from Ru5P to RuBP. Thus we have a cycle, explaining the other part of the name 'Calvin cycle'. While most of these are bona fide reactions, that leading from GAP to Ru5P is an effective reaction (or, less respectfully, a fudge reaction) resulting from collapsing part of a more complicated network. This can easily be recognized by means of the exotic stoichiometry, with five molecules going in and three coming out. In addition there are two transport processes in which PGA and GAP are exported to the cytosol from the chloroplast where the Calvin cycle takes place. Thus some simple models of photosynthesis (to be considered in more detail in the next section) have five species and seven reactions.

In this paper there is no attempt to present a systematic catalogue of models. Instead it is like an accompanied walk through a zoo, where the visitor is taken to see the lions and the elephants but also less familiar exhibits such as the giant anteater or the Tasmanian devil. It starts with the simplest and best known models and is led by the consideration of various issues to ones which have been studied less.

So many related models are considered in what follows that it would be cumbersome to have a name for each of them. Some names will be used but in addition the models will be given numbers according to the pattern Model  $m.n.k$ , where roughly speaking this means the variant  $k$  of the model  $n$  first introduced in section  $m$ .

The paper is organized as follows. Section 2 introduces the models with five species and is mainly a survey of known results concerning them. These provide information about the existence and stability of positive steady states and the existence of solutions where the concentrations tend to zero or to infinity at late times. The process of passing from one model to another by making an internal species into an external one is carried out in a simple example. In Section 3 models with a larger number of variables (about fifteen) are considered which are variants of one introduced in [14]. Some known results on the (non)-existence of positive steady states and the ways in which concentrations can approach zero at late times are reviewed and extended. It is shown how the model of [14] itself can be given a clear mathematical formulation as a system of DAE. It is also shown how ATP can be made into an external species in these models. Section 4 contains a proof that the model of [14] admits more than one positive steady state for suitable values of the parameters. This is related to a stoichiometric generator for the network and other generators, which might also be helpful in the search for steady states, are presented. A similar approach can be applied to the related reduced Poolman model and this is done in Section 5, where it is shown that there are parameter values for which there exist at least three positive steady states. The last section is concerned with some prospects for future progress and briefly discusses some simplified models due to Hahn.

## 2 The five-species models

In this section some models will be considered where the unknowns are the concentrations of the five substrates introduced in the previous section. The concentration of a substance  $X$  is denoted by  $x_X$ . The reactions are those mentioned in the introduction and they are all assumed to be irreversible. The simplest type of kinetics which can be assumed is mass action kinetics. For this we need to know the exact stoichiometry. At this point there is an ambiguity resulting from the fudge reaction. In that case the biochemistry does not determine unique values for the stoichiometric coefficients. It only determines the ratio of the number of molecules going into the reaction and the number coming out. One possible choice, which was made in [19], is to use the coefficients 1 and 0.6. Since this is only an effective reaction there is no strong argument that these coefficients should be integers. Nevertheless in [6] the authors preferred to use 5 and 3. This does make a difference to the evolution equations resulting from the assumption of mass action kinetics. With the stoichiometry of [6] mass action kinetics leads to nonlinear evolution equations (Model 2.1.1) while the kinetics of [19] gives linear equations (Model 2.1.2). An alternative to mass action kinetics is Michaelis-Menten kinetics, either with the coefficients of [6] (Model 2.2.1) or those of [19] (Model 2.2.2).

We now examine the dynamics of these models. It was shown in [6] that if the reaction constants satisfy a certain inequality ( $k_2 \leq 5k_6$ ) Model 2.1.1 does not possess any positive steady states while if  $k_2 > 5k_6$  it possesses precisely one positive steady state for given values of the parameters. These statements are obtained by explicit calculation. In the latter case it was shown that the steady state is unstable. This could be seen as a disappointment since it might be supposed for biological reasons that the cycle can exist in a stable configuration. This is not absolutely clear since a good model need not give a good description of the dynamics globally in time but only on a time scale long enough so as to capture the processes which are to be described. This quantitative line of thought will not be pursued further here. In [17] it was further shown that in the case  $k_2 \leq 5k_6$  all concentrations tend to zero as time tends to infinity. This was done with the help of a Lyapunov function. In [6] and [17] the inequality relating  $k_2$  and  $k_6$  was not given any biological interpretation and no explanation was given for the Lyapunov function which was found by trial and error. More insight on these questions was obtained while studying a more complicated model of the Calvin cycle in [12]. The reaction constant  $k_6$  controls the rate of export of PGA from the chloroplast and in reality this is coupled to the import of inorganic phosphate. Thus intuitively  $k_6$  secretly contains a dependence on the constant concentration of inorganic phosphate in the cytosol. The positive steady state disappears when there is too much phosphate in the cytosol. Then the production of sugars by the cycle cannot keep up with the export. This phenomenon has been called overload breakdown [14]. To obtain some insight into the Lyapunov function it is helpful to consider the total number of carbon atoms in the system. The reactions within the cycle conserve carbon apart from the fact that at one point carbon dioxide is imported. The export processes also

do not conserve carbon. Nevertheless the time derivative of the total amount of carbon only has a few contributions. One of them has a positive sign but modifying the coefficient of  $x_{\text{PGA}}$  allows a Lyapunov function  $L_1$  to be obtained. There remains the question of what happens to solutions of Model 2.1.1 at late times when  $k_2 > 5k_6$ . It was shown in [17] that there are solutions where all concentrations tend to zero at late times (using a modification  $L_2$  of  $L_1$ ) and solutions for which the concentrations tend to infinity as  $t \rightarrow \infty$  (runaway solutions) and their leading order asymptotics were determined. From what has just been said it can be seen that a number of facts are known about the dynamics of Model 2.1.1 but there remain open questions, for instance whether periodic solutions exist. Information about Model 2.1.2 has also been obtained in [17]. In particular, there are either no positive steady states or a whole continuum of steady states, depending on the values of the reaction constants. Both solutions converging to the origin at late times and runaway solutions occur.

When mass action kinetics is replaced by Michaelis-Menten kinetics there are still runaway solutions but there are also more interesting steady states. Concerning Model 2.2.2 it stated in [19] that there is at most one steady state which is ‘physiologically feasible’. This last condition includes restrictions on the values of the model parameters. In some cases a parameter interval is chosen centred at a value taken from the experimental literature. Some Michaelis constants  $K_{mi}$  are set to fixed values and it is not clear to this author where these values come from. The paper [19] uses computer-assisted methods which are claimed to prove the assertion about the limitation on steady states. In [5] a purely analytical proof of the assertion was given under the assumptions on the Michaelis constants made in [19]. It was also proved that the assertion depends essentially on these assumptions. There are examples with  $\kappa = (K_{m7} - K_{m4})(K_{m6} - K_{m21}) < 0$  for which there exist two distinct positive steady states and if both factors in the expression for  $\kappa$  vanish there is a continuum of steady states. It was also shown that there exist cases with two isolated steady states where one of them is stable and the other is unstable. This is proved by showing that there is a bifurcation with a one-dimensional centre manifold. In particular we obtain models admitting a stable positive steady state although it is unclear whether the parameter values required for this are biologically reasonable. Model 2.2.1 also permits the existence of two positive steady states, one of which is stable and the other is unstable.

Another type of model, considered in [6], is obtained if each of the basic reactions considered up to now is replaced by a Michaelis-Menten scheme with a substrate, an enzyme and a substrate-enzyme complex (Models 2.3.1 and 2.3.2) and these will be discussed in this section although they contain many more than five variables. It is possible to pass from these models to Models 2.2.1 and 2.2.2 by a Michaelis-Menten reduction which is well-behaved in the sense of geometric singular perturbation theory (GSPT) - the transverse eigenvalues have negative real parts. (For background information on GSPT we refer to [11].) This means that we can transfer information on the existence and stability of steady states from Models 2.2.1 and 2.2.2 to Models 2.3.1 and 2.3.2 in a straightforward way.

In fact the existence of more than one steady state of Model 2.3.1 was discovered directly in [6] with the help of elementary flux modes.

In another model introduced in [6] ATP was made into an internal species, thus producing a six-variable model and diffusion of ATP was included. Restricting consideration to spatially homogeneous solutions reduces the resulting system of reaction-diffusion equations to a system of ODE (Model 2.4.1). It turns out that Model 2.4.1 can be analysed as in the cases of Models 2.2.1 and 2.2.2, giving the existence of two steady states, one stable and one unstable [5]. Interestingly, the solutions of Model 2.4.1 are bounded although this is non-trivial to prove [17]. In all these models  $\omega$ -limit points where some concentration vanishes are strongly restricted. In Models 2.1.1, 2.1.2, 2.2.1 and 2.2.2 the only point which can occur is the origin. In Model 2.3.1 the analogue of this is a situation where all substrates are exhausted and the enzymes are completely in the unbound form. In Model 2.4.1 the corresponding situation is that all concentrations except that of ATP are zero and the concentration of ATP takes on its maximal value.

The process of making a species into an external species can be illustrated by showing how Model 2.1.1 can be obtained as a limit of Model 2.4.1. The evolution equations of Model 2.4.1 are

$$\frac{dx_{\text{RuBP}}}{dt} = k_5 x_{\text{Ru5P}} x_{\text{ATP}} - k_1 x_{\text{RuBP}}, \quad (1)$$

$$\frac{dx_{\text{PGA}}}{dt} = 2k_1 x_{\text{RuBP}} - k_2 x_{\text{PGA}} x_{\text{ATP}} - k_6 x_{\text{PGA}}, \quad (2)$$

$$\frac{dx_{\text{DPGA}}}{dt} = k_2 x_{\text{PGA}} x_{\text{ATP}} - k_3 x_{\text{DPGA}}, \quad (3)$$

$$\frac{dx_{\text{GAP}}}{dt} = k_3 x_{\text{DPGA}} - 5k_4 x_{\text{GAP}}^5 - k_7 x_{\text{GAP}}, \quad (4)$$

$$\frac{dx_{\text{Ru5P}}}{dt} = -k_5 x_{\text{Ru5P}} x_{\text{ATP}} + 3k_4 x_{\text{GAP}}^5, \quad (5)$$

$$\frac{dx_{\text{ATP}}}{dt} = -k_2 x_{\text{PGA}} x_{\text{ATP}} - k_5 x_{\text{Ru5P}} x_{\text{ATP}} + k_8 (c_A - x_{\text{ATP}}) \quad (6)$$

where the constant  $c_A$  is the total concentration of adenosine phosphates. We would now like to consider a situation where ATP is in excess. Equivalently we can consider a situation where the concentrations of all substances except ATP are very small. Define  $x_X = \eta \tilde{x}_X$  for each substance X except ATP. Define  $\tilde{k}_4 = \eta^4 k_4$ . Then making these substitutions gives

$$\frac{d\tilde{x}_{\text{RuBP}}}{dt} = k_5 \tilde{x}_{\text{Ru5P}} x_{\text{ATP}} - k_1 \tilde{x}_{\text{RuBP}}, \quad (7)$$

$$\frac{d\tilde{x}_{\text{PGA}}}{dt} = 2k_1 \tilde{x}_{\text{RuBP}} - k_2 \tilde{x}_{\text{PGA}} x_{\text{ATP}} - k_6 \tilde{x}_{\text{PGA}}, \quad (8)$$

$$\frac{d\tilde{x}_{\text{DPGA}}}{dt} = k_2 \tilde{x}_{\text{PGA}} x_{\text{ATP}} - k_3 \tilde{x}_{\text{DPGA}}, \quad (9)$$

$$\frac{d\tilde{x}_{\text{GAP}}}{dt} = k_3 \tilde{x}_{\text{DPGA}} - 5\tilde{k}_4 \tilde{x}_{\text{GAP}}^5 - k_7 \tilde{x}_{\text{GAP}}, \quad (10)$$

$$\frac{d\tilde{x}_{\text{Ru5P}}}{dt} = -k_5\tilde{x}_{\text{Ru5P}}x_{\text{ATP}} + 3\tilde{k}_4\tilde{x}_{\text{GAP}}^5, \quad (11)$$

$$\frac{d\tilde{x}_{\text{ATP}}}{dt} = -\eta k_2\tilde{x}_{\text{PGA}}x_{\text{ATP}} - \eta k_5\tilde{x}_{\text{Ru5P}}x_{\text{ATP}} + k_8(c_A - x_{\text{ATP}}). \quad (12)$$

Letting  $\eta$  tend to zero gives a system for which  $x_{\text{ATP}} = c_A$  is an invariant manifold and the restriction of the system to that manifold reproduces the equations of Model 2.1.1. This is a regular limit and it follows from the existence of an unstable hyperbolic positive steady state in the limiting system that Model 2.4.1 also has an unstable hyperbolic positive steady state. Note that the perturbed steady state for  $\eta$  small and positive does satisfy  $x_{\text{ATP}} < c_A$  since otherwise equation (12) would lead to a contradiction. For this system the information about a steady state obtained by the perturbation argument is less than what is already known by analysing the full system directly. The argument has nevertheless been presented here since analogous arguments may be useful for obtaining information about more complicated systems where no alternative is available.

### 3 The Pettersson model and modifications of it

The models considered in this section involve more unknowns than those of the previous section. The starting point is a model introduced in a paper by Pettersson and Ryde-Pettersson [14] which we refer to for brevity as the Pettersson model. The substances included are roughly speaking those which Calvin found to appear after the dark reactions had run for a few minutes. In addition to those in the five-variable models these are dihydroxyacetone phosphate (DHAP), fructose 1,6-bisphosphate (FBP), fructose 6-phosphate (F6P), erythrose 4-phosphate (E4P), sedoheptulose 7-phosphate (S7P), sedoheptulose 1,7-bisphosphate (SBP), xylulose 5-phosphate (X5P) and ribose 5-phosphate (R5P). In addition the process by which sugars can be stored in the chloroplast as starch is included. Starch itself is treated as an external species. The intermediates glucose 6-phosphate (G6P) and glucose 1-phosphate (G1P) are included as internal species. In contrast to the models of the previous section inorganic phosphate in the chloroplast,  $P_i$ , is modelled dynamically, as is ATP. On the other hand NADPH is still treated as an external species. Some of the reactions which were treated as irreversible in the models of the previous section are treated as reversible in the Pettersson model, for instance the reaction interconverting PGA and DPGA. The decision, which reactions to treat as reversible and which as irreversible in the Pettersson model is based on experimental data. The only reactions treated as irreversible are those whose substrates are Ru5P, RuBP, FBP and G1P together with the transport processes to the cytosol and to starch.

The Pettersson model (Modell 3.1.1) will now be described. In [14] the time derivatives of the relevant concentrations are expressed in terms of the rates  $v_i$  of the different reactions. The equations are

$$\frac{dx_{\text{RuBP}}}{dt} = v_{13} - v_1, \quad (13)$$

$$\frac{dx_{\text{PGA}}}{dt} = 2v_1 - v_2 - v_{\text{PGA}}, \quad (14)$$

$$\frac{dx_{\text{DPGA}}}{dt} = v_2 - v_3, \quad (15)$$

$$\frac{dx_{\text{ATP}}}{dt} = v_{16} - v_2 - v_{13} - v_{\text{st}}, \quad (16)$$

$$\frac{dx_{\text{GAP}}}{dt} = v_3 - v_4 - v_5 - v_7 - v_{10} - v_{\text{GAP}}, \quad (17)$$

$$\frac{dx_{\text{DHAP}}}{dt} = v_4 - v_5 - v_8 - v_{\text{DHAP}}, \quad (18)$$

$$\frac{dx_{\text{FBP}}}{dt} = v_5 - v_6, \quad (19)$$

$$\frac{dx_{\text{F6P}}}{dt} = v_6 - v_7 - v_{14}, \quad (20)$$

$$\frac{dx_{\text{E4P}}}{dt} = v_7 - v_8, \quad (21)$$

$$\frac{dx_{\text{X5P}}}{dt} = v_7 + v_{10} - v_{12}, \quad (22)$$

$$\frac{dx_{\text{SBP}}}{dt} = v_8 - v_9, \quad (23)$$

$$\frac{dx_{\text{S7P}}}{dt} = v_9 - v_{10}, \quad (24)$$

$$\frac{dx_{\text{R5P}}}{dt} = v_{10} - v_{11}, \quad (25)$$

$$\frac{dx_{\text{Ru5P}}}{dt} = v_{11} + v_{12} - v_{13}, \quad (26)$$

$$\frac{dx_{\text{G6P}}}{dt} = v_{14} - v_{15}, \quad (27)$$

$$\frac{dx_{\text{G1P}}}{dt} = v_{15} - v_{\text{st}}, \quad (28)$$

$$\frac{dx_{\text{P}_i}}{dt} = v_3 + v_6 + v_9 + v_{\text{PGA}} + v_{\text{GAP}} + v_{\text{DHAP}} + 2v_{\text{st}} - v_{16}. \quad (29)$$

The total amount of phosphate in the chloroplast is a conserved quantity and may be used to eliminate the concentration of inorganic phosphate in the chloroplast from the equations in favour of the other variables. It is assumed that the reversible reactions are much faster than the irreversible ones. This can be implemented mathematically by introducing a small parameter  $\epsilon$  and defining  $\tilde{v}_i = \epsilon v_i$  for the fast reactions. The slow reactions, whose rates are not rescaled, are those with reaction rates  $v_1, v_6, v_9, v_{13}, v_{16}, v_{\text{PGA}}, v_{\text{GAP}}, v_{\text{DHAP}}$  and  $v_{\text{st}}$ . For each of the slow reactions an explicit phenomenological expression is given for the rate. This incorporates the known experimental facts on the activation and inhibition of certain reactions due to the influence of other substances. No expressions are given for the rates of the fast reactions. Instead it is assumed that these reactions can be taken to be in equilibrium, which gives algebraic equations relating the concentrations. It will be shown below how this can be



implemented mathematically.

At this point we interrupt the discussion of the Pettersson model and instead take the reaction network underlying the Pettersson model including its stoichiometry and apply mass action kinetics to get something which was called the Pettersson-MA model in [12] (Model 3.2.1). The strategy adopted in [12] was to study the dynamics of Model 3.2.1 so as to try to obtain some insights for tackling the Pettersson model later. There is a related model with an additional reaction which liberates G1P from starch (Model 3.2.2). There the above evolution equations are modified by adding a contribution  $v_{17}$  to the evolution for  $x_{\text{G1P}}$  and a contribution  $-v_{17}$  to the evolution equation for  $x_{\text{P}_1}$ . This will be called the Poolman-MA model since a modification of the Pettersson model including a mechanism of this type was first introduced by Poolman [15]. Poolman himself used the same reaction rates as in [14] for the slow reactions and treated the liberation of G1P as a slow reaction while taking mass action kinetics for the fast reactions. We call the resulting system of ODE the Poolman model (Model 3.1.2). A hybrid model can be obtained by taking the reactions included in the Pettersson model with the reaction rates as in the Poolman model (Model 3.3.1). This is obtained from the Poolman model by setting one of the reaction constants  $k_{32}$  to zero. In these models the total amount of phosphate is conserved and every unknown contains some phosphate. Thus the conservation law implies that all solutions are bounded and runaway solutions are ruled out for these models. It was already mentioned that the export of sugars from the chloroplast is coupled to the import of inorganic phosphate (whose concentration in the cytosol is assumed constant in the model). It is indicated in [14] that if the external concentration of phosphate is too high then no positive steady state will exist. This is the phenomenon of overload breakdown. Poolman suggested that overload breakdown could be avoided by introducing the release of G1P from starch. In the case of Model 3.2.1 it was shown in [12] that if  $k_3 c_A \leq 5k_{28}$  there exists a Lyapunov function related to the function  $L_1$  of the last section and this proves that under this condition Model 3.2.1 has no positive steady states. Here the  $k_i$  are reaction constants and  $c_A$  is the total concentration of adenosine phosphates. For Model 3.2.2 this construction no longer works. When  $k_3 c_A > 5k_{28}$  in Model 3.2.1 it is possible to construct an analogue of the function  $L_2$  of the last section which gives the conclusion that there exist no positive steady states where  $L_2$  is less than a certain number depending only on the reaction constants. Here we define

$$L_2 = L_1 - \frac{1}{2}(x_{\text{DPGA}} + x_{\text{GAP}} + x_{\text{DHAP}}). \quad (30)$$

and it satisfies

$$\begin{aligned} \frac{d(5L_2)}{dt} = & \frac{1}{2} (2k_9 x_{\text{DHAP}} x_{\text{GAP}} + k_{12} x_{\text{FBP}} x_{\text{GAP}} + k_{14} x_{\text{E4P}} x_{\text{DHAP}} \\ & + k_{17} x_{\text{S7P}} x_{\text{GAP}} - 5k_{29} x_{\text{GAP}} - 5k_{30} x_{\text{DHAP}} - k_{11} x_{\text{E4P}} x_{\text{X5P}} \\ & - k_{16} x_{\text{X5P}} x_{\text{R5P}} - 5x_{\text{PGA}} - k_8 x_{\text{FBP}} - k_{13} x_{\text{SBP}}). \end{aligned} \quad (31)$$

When  $L_2$  is sufficiently small the positive terms on the right hand side are

dominated by the negative ones. Information can also be obtained for Model 3.3.1 by using the function  $L_1$ . In that case  $L_1$  is decreasing provided the quantity  $\frac{1}{2}k_3x_{\text{ATP}} - \frac{5}{2}v_{\text{PGA}}$  is negative. Now  $v_{\text{PGA}} = \frac{V_{\text{ex}}x_{\text{PGA}}}{NK_{\text{PGA}}}$  where

$$N = 1 + \left(1 + \frac{K_{\text{P}_{\text{ext}}}}{x_{\text{P}_{\text{ext}}}}\right) \left(\frac{x_{\text{P}_i}}{K_{\text{P}_i}} + \frac{x_{\text{PGA}}}{K_{\text{PGA}}} + \frac{x_{\text{PGA}}}{K_{\text{GPA}}} + \frac{x_{\text{DHAP}}}{K_{\text{DHAP}}}\right) \quad (32)$$

and the other quantities which have not previously been defined are positive constants. Here we treat the total amount of phosphate as a parameter and then if  $k_3$  is chosen small enough for fixed values of the other parameters in the kinetics we get a positive lower bound for  $x_{\text{PGA}}^{-1}v_{\text{PGA}}$ . Thus under these conditions  $L_1$  is decreasing. In particular Model 3.3.1 has no positive steady states when the parameters are restricted in this way.

In [12] conditions were derived for  $\omega$ -limit points of positive solutions of Models 3.2.1 and 3.2.2. It was pointed out in [12] that many of the arguments used apply to the original Poolman model since the only property of the reaction rates which is used is under what circumstances they are positive or zero and this is not changed when the mass action kinetics is replaced by the more complicated kinetics of the Poolman model. The same argument applies to the hybrid model. It thus follows from the arguments in [12] that Models 3.1.2, 3.2.1, 3.2.2 and 3.3.1 have the property that the only substances whose concentrations may fail to vanish at an  $\omega$ -limit point of a positive solution where at least one concentration vanishes are G1P, G6P, F6P, E4P, S7P and  $\text{P}_i$ .

In [12] information was also obtained on how these points may be approached by positive solutions of Models 3.2.1 and 3.2.2. This is done by linearizing about steady states where some concentrations are zero and analysing the eigenvalues of the linearization. In some cases spectral stability could be determined but other cases remain open. In many cases where the spectral analysis was successful it turned out that the centre manifold coincides with the center subspace and the qualitative behaviour on the centre manifold could be analysed. In other cases the centre manifold is nonlinear and its Taylor expansion not yet been computed.

Consider now again the evolution equations for the Pettersson model expressed in terms of the reaction rates  $v_i$ . In [14] five linear combinations  $y_i$  of concentrations are identified whose time derivatives only depend on the slow reaction rates. Suppose we now complement these by a suitable set of concentrations  $z_i$ , for instance all those except  $x_{\text{RuBP}}$ ,  $x_{\text{F6P}}$ ,  $x_{\text{Ru5P}}$ ,  $x_{\text{DHAP}}$  and  $x_{\text{ATP}}$ , which we denote by  $s_i$ . Then the concentrations of the internal species are related to the variables  $y_i$  and  $z_i$  by an invertible linear transformation. Consider the equations of the hybrid model, expressed in terms of the variables  $y_i$  and  $z_i$ . If we write them in terms of the  $\tilde{v}_i$  then all the terms on the right hand side of the evolution equations for the  $y_i$  are regular in  $\epsilon$  while many of those on the right hand side of the equations for the  $z_i$  contain a factor  $\epsilon^{-1}$ . Multiplying these equations with  $\epsilon$  and letting  $\epsilon$  tend to zero gives a system of algebraic equations. When expressed in terms of the  $\tilde{v}_i$  these equations are linear and they imply that the  $\tilde{v}_i$  vanish for all the fast reactions at  $\epsilon = 0$ . This fact can be

read off from the subnetwork obtained by deleting the slow reactions from the full Pettersson network. Now the  $\tilde{v}_i$  can be obtained from  $v_i$  by replacing the reaction constants  $k_i$  by  $\tilde{k}_i = \epsilon k_i$ . If the  $\tilde{k}_i$  are chosen independent of  $\epsilon$  the algebraic equations (20)-(30) of [14] for the concentrations are obtained. Thus in the limit  $\epsilon \rightarrow 0$  the hybrid model becomes a system consisting of the differential equations (48) and the algebraic equations (20)-(30) of [14]. It defines a system of DAE for the variables  $y_i$  and  $z_i$ . This is what we refer to as the Pettersson model. Without further information it is not even clear that local existence holds for this system. In [14] it is claimed that the equations (20)-(30) of that paper can be used to obtain an explicit closed system of evolution equations for the variables  $s_i$  but the calculations presented there are not complete. A similar reduction to a DAE can be carried out for the Poolman model and we call the result the reduced Poolman model (Model 3.3.2).

Any of the models considered in this section may be modified so as to make ATP and inorganic phosphate external species. By analogy with what was done in the case of Model 2.4.1 we can fix the concentration of ATP and  $c_A$  and rescale the concentrations of the other substances by a factor  $\eta$ . The fact of having eliminated the concentration of inorganic phosphate by using the conservation of the total amount of phosphate means that  $x_{P_i}$  then automatically becomes constant. In the same way as  $k_4$  had to be scaled by a power of  $\eta$  in Model 2.4.1 it is necessary to rescale the reaction constants in the reactions with two substrates in order to get a non-zero limit. For instance, using the notation of [12] we introduce  $\tilde{k}_9 = \eta k_9$ . The other reaction constants which should be rescaled in a similar manner are  $k_{11}$ ,  $k_{12}$ ,  $k_{14}$ ,  $k_{16}$  and  $k_{17}$ . To maintain the non-trivial effects of saturation, activation and inhibition in the slow reactions it is also necessary to rescale certain Michaelis constants. The constants involved are  $K_{m6}$ ,  $K_{i61}$ ,  $K_{m9}$ ,  $K_{mst1}$ ,  $K_{m1}$ ,  $K_{i11}$ ,  $K_{i12}$ ,  $K_{i13}$ ,  $K_{i15}$ ,  $K_{m131}$ ,  $K_{i131}$ ,  $K_{i132}$ ,  $K_{ast1}$ ,  $K_{ast2}$ ,  $K_{ast3}$ ,  $K_{PGA}$ ,  $K_{GAP}$  and  $K_{DHAP}$ . Let us call the results of modifying Models 3.1.1 and 3.3.1 in this way Models 3.4.1 and 3.4.2 respectively. In both of these cases the modified model has an invariant manifold  $x_{ATP} = c_A$  for  $\eta = 0$  and the restriction of the system to that submanifold reproduces the model with ATP as an external species. As in the discussion of Model 2.4.1 in the last section this means that any hyperbolic positive steady state of Model 3.4.1 or 3.4.2 gives rise to a hyperbolic positive steady state of Model 3.1.1 or 3.3.1, respectively. Thus information on steady states can be obtained from information on the corresponding models with the concentrations of ATP and  $P_i$  frozen.

## 4 Steady states of the Pettersson model

Five of the equations in the Pettersson model are evolution equations. The right hand sides of these equations are the functions  $F_i$  in the equations (54)-(58) of [14] and their vanishing is equivalent to the equations (42)-(47) of [14], which are, with the definition  $v = v_1$ ,

$$v_1 = v, \tag{33}$$

$$v_6 = \frac{v}{3} + v_{\text{st}}, \quad (34)$$

$$v_9 = \frac{v}{3}, \quad (35)$$

$$v_{13} = v, \quad (36)$$

$$v_{16} = 3v + v_{\text{st}} - v_{\text{PGA}}, \quad (37)$$

$$v = 3v_{\text{ex}} + 6v_{\text{st}} \quad (38)$$

where  $v_{\text{ex}} = v_{\text{PGA}} + v_{\text{GAP}} + v_{\text{DHAP}}$ . In this section we concentrate on Model 3.4.1, where all these equations hold except that containing  $v_{16}$ . Suppose that  $x_{\text{DHAP}}$  is given. Then  $\tilde{v}_4 = 0$  fixes the value of  $x_{\text{GAP}}$ . Then  $\tilde{v}_3 = 0$  and  $\tilde{v}_2 = 0$  fix the values of  $x_{\text{DPGA}}$  and  $x_{\text{PGA}}$ . In addition  $\tilde{v}_5 = 0$  fixes the value of  $x_{\text{FBP}}$ . With the information we have it is possible to compute  $v_{\text{ext}}$  in terms of  $x_{\text{DHAP}}$ . Suppose now that  $v_{\text{st}}$  is also fixed. Then with this information it is possible to obtain  $v$  and hence  $v_9$  and  $v_{13}$ . The quantities  $x_{\text{SBP}}$ ,  $x_{\text{RuBP}}$  and  $x_{\text{Ru5P}}$  are then uniquely determined. To ensure the existence of these quantities it suffices to assume that the parameters  $V_9$ ,  $V_{13}$  and  $V_1$  are large enough. The equations  $\tilde{v}_{11} = 0$  and  $\tilde{v}_{12} = 0$  fix  $x_{\text{R5P}}$  and  $x_{\text{X5P}}$ . Next the equation  $\tilde{v}_8 = 0$  allows  $x_{\text{E4P}}$  to be determined. Then  $\tilde{v}_7 = 0$  can be used to determine  $x_{\text{F6P}}$  and  $\tilde{v}_{14} = 0$  and  $\tilde{v}_{15} = 0$  give  $x_{\text{G6P}}$  and  $x_{\text{G1P}}$ . This leaves two consistency conditions, namely the equation for  $v_6$  and the expression for  $v_{\text{st}}$  in terms of  $x_{\text{G1P}}$ . Let these be denoted abstractly by  $\Phi_1(x_{\text{DHAP}}, v_{\text{st}}) = 0$  and  $\Phi_2(x_{\text{DHAP}}, v_{\text{st}}) = 0$  respectively.

A general strategy for looking for positive steady states of the evolution equations defined by a given network is to look at limiting cases where some of the reaction constants are set to zero and the smaller network obtained by discarding the reactions concerned. Trying to do this for a larger network without some guiding principles may fail because there are too many possibilities. A concept which can be used as a guiding principle is that of elementary flux modes. There is a theory of how to produce steady states using these objects [4] but an alternative is to use elementary flux mode to guess which reaction constants to set to zero and then proceed directly with the construction of steady states. This possibility was used in [17] to give an existence proof of steady states of Model 2.3.1 and it will also be applied in what follows. The elementary flux modes computed are not required for the proofs themselves but they help to put those proofs into context. They helped to find the proofs and this approach might also turn out to be useful for analysing other similar models in the future.

Consider the equations satisfied by the fluxes in steady states of a model defined by the Pettersson network. These are

$$2v_1 - v_2 - v_{\text{PGA}} = 0, \quad (39)$$

$$v_2 = v_3, \quad (40)$$

$$v_3 - v_4 - v_5 - v_7 - v_{10} - v_{\text{GAP}} = 0, \quad (41)$$

$$v_4 - v_5 - v_8 - v_{\text{DHAP}} = 0, \quad (42)$$

$$v_5 = v_6, \quad (43)$$

$$v_6 - v_7 - v_{14} = 0, \quad (44)$$

$$v_7 = v_8, \quad (45)$$

$$v_8 = v_9, \quad (46)$$

$$v_9 = v_{10}, \quad (47)$$

$$v_7 + v_{10} - v_{12} = 0, \quad (48)$$

$$v_{10} = v_{11}, \quad (49)$$

$$v_{11} + v_{12} - v_{13} = 0, \quad (50)$$

$$v_{13} = v_1, \quad (51)$$

$$v_{14} = v_{15}, \quad (52)$$

$$v_{15} = v_{\text{st}}, \quad (53)$$

$$v_{16} - v_2 - v_{13} - v_{\text{st}} = 0. \quad (54)$$

The solutions of these linear equations can be parametrized with the help of stoichiometric generators. The relevant terminology will now be recalled (cf. [4]). The system of ODE arising from a reaction network can be written in the form  $\dot{x} = Nv(x)$ , where  $N$  is the stoichiometric matrix and  $v(x)$  are the reaction rates. In this context reversible reactions are treated as two separate reactions. This means that there are two columns of  $N$  corresponding to each reversible reaction. The column corresponding to the forward reaction is minus the column corresponding to the backward reaction. If we discard one of the two columns corresponding to each reversible reaction a truncated matrix  $\bar{N}$  is obtained. The kernels of the matrices  $N$  and  $\bar{N}$  are related in a simple way which will be described below. The set of reaction rates at a steady state is an element of the kernel of  $N$  with non-negative components. We can think of this as a point in the space of real-valued functions on the set  $\mathcal{R}$  of reactions. The set of all non-negative elements of the kernel of  $N$  is a positive cone and thus consists of all vectors of the form  $\sum_i a_i w_i$  with  $a_i$  non-negative coefficients and  $w_i$  a finite number of vectors which in this context are called elementary flux modes [18], [4]. Each of these vectors has the property that setting some but not all of its components to zero gives a vector which is not in the kernel of  $N$ . Another important quantity is the incidence matrix. It has one row for each complex (quantity on the left or right hand side of a reaction) and one column for each reaction. The element for the left hand side of the reaction is  $-1$ , the element for the right hand side is  $+1$  and all other elements are zero. A vector which is in the kernel of the incidence matrix is in the kernel of  $N$ . Elementary flux modes which are not in the kernel of the incidence matrix are called stoichiometric generators. For a reversible reaction let us make a choice of which is the forward direction, so as to get a forward reaction  $r_+$  and a backward reaction  $r_-$ . Then the vector which has the components  $+1$  at  $r_+$ ,  $-1$  at  $r_-$  and all other components zero belongs to the kernel of the incidence matrix. Let us call this a trivial mode. It is an elementary flux mode which is not a stoichiometric generator. The kernel of  $N$  is the joint span of the kernel of  $\bar{N}$  and the trivial modes.

In the case of the Pettersson network the following vectors with components

$w_i$  are stoichiometric generators

$$[3 \ 6 \ 6 \ 3 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 2 \ 3 \ 0 \ 0 \ 9 \ 0 \ 0 \ 1 \ 0], \quad (55)$$

$$[3 \ 6 \ 6 \ 2 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 2 \ 3 \ 0 \ 0 \ 9 \ 0 \ 1 \ 0 \ 0], \quad (56)$$

$$[3 \ 5 \ 5 \ 2 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 2 \ 3 \ 0 \ 0 \ 8 \ 1 \ 0 \ 0 \ 0], \quad (57)$$

$$[6 \ 12 \ 12 \ 5 \ 3 \ 3 \ 2 \ 2 \ 2 \ 2 \ 2 \ 2 \ 4 \ 6 \ 1 \ 1 \ 19 \ 0 \ 0 \ 0 \ 1]. \quad (58)$$

Here the components are written in the order

$$[v_1 \ v_2 \ v_3 \ v_4 \ v_5 \ v_6 \ v_7 \ v_8 \ v_9 \ v_{10} \ v_{11} \ v_{12} \ v_{13} \ v_{14} \ v_{15} \ v_{16} \ v_{\text{PGA}} \ v_{\text{GAP}} \ v_{\text{DHAP}} \ v_{\text{st}}]. \quad (59)$$

We only write out the components corresponding to the forward reactions. In other words these vectors belong to the kernel of  $\bar{N}$ . To get the full mode, which is an element of the kernel of  $N$ , it would be necessary to add zeroes for the backward reactions. It is easily checked that the vectors defined by (55)-(58) are solutions of the equations (39)-(54). It can also be shown that any solution for which the last four components are zero is zero. Furthermore, any non-negative solution for which at least one of the last four components is non-zero is a linear combination of the four solutions above and hence all the components except the last four are zero. This verifies the defining property of elementary flux modes that a vector obtained by setting some, but not all, of the components of the generator to zero is not a solution. These vectors do not belong to the kernel of the incidence matrix and hence are stoichiometric generators. Finally, all solutions are linear combinations with non-negative coefficients of these generators. This follows from the facts that the generators are linearly independent and that the dimension of the kernel of  $\bar{N}$  is four. Each of the generators is obtained by shutting off all but one of the output reactions.

Each stoichiometric generator defines a subnetwork obtained by setting those reaction rates to zero for which the corresponding component of the generator is zero. If mass action kinetics are being considered the desired reaction rates can be set to zero by setting the corresponding reaction constants to zero. For other kinetics some more thought is necessary. In most of the slow reactions we can set  $v_i$  to zero by setting the corresponding coefficient  $V_i$  to zero. The exceptions are the transport reactions to the cytosol. There we have three reaction rates but only one coefficient  $V_{\text{ex}}$ . Here the desired reaction rates,  $v_X$  can be set to zero by setting  $K_X^{-1}$  formally to zero. In other words, in the limit certain constants  $K_X$  tend to infinity. It also turns out to be helpful to set the quantity  $K_{i61}^{-1}$  to zero in the limit. If we make the same assumptions on the kinetics as in the Pettersson model we get a system of DAE corresponding to the subnetwork. Call the system of this type obtained from the first of the four generators listed above Model 4.1.1. Concretely, it is obtained from the Pettersson model by setting the reaction constants  $k_{23}$ ,  $k_{24}$ ,  $k_{25}$ ,  $k_{26}$ ,  $k_{28}$  and  $k_{29}$  to zero and discarding the variables  $x_{\text{G1P}}$ ,  $x_{\text{G6P}}$ . A limit is considered where these reaction constants are multiplied by a small constant  $\zeta$  and the constants  $K_{\text{PGA}}$  and  $K_{\text{GAP}}$  and  $K_{i61}$  are multiplied by  $\zeta^{-1}$ . Biologically this corresponds to a situation where PGA and GAP not only fail to be exported

but even fail to bind to the transporter and thus do not compete with DHAP. In a similar way it is possible to obtain an analogue of the hybrid model for the subnetwork. Call it Model 4.2.1. We can freeze the concentrations of ATP and  $P_i$  in Models 4.1.1 and 4.2.1 to get Models 4.1.2 and 4.2.2. Model 4.1.2 is close to a modelling approach used in [13] although in that paper no complete system of equations was written out. The steady states of Model 4.1.2 can be studied by following calculations in [13]. There are two differences between Model 4.1.2 and the situation in [13]. One of these corresponds to setting the coefficients  $K_{i62}^{-1}$  to zero in the expression for  $v_6$  in [14] while the other has to do with the fact that  $v_{\text{PGA}}$  and  $v_{\text{GAP}}$  are absent from Model 4.1.2.

The equations (33)-(38) for the reaction rates in the Pettersson model are modified in the subnetwork by the removal of  $v_{\text{st}}$  and  $v_{\text{PGA}}$ . For  $\zeta = 0$  we have  $\Phi_2 = v_{\text{st}}$ . Following the computations done above we find that for  $\zeta = 0$

$$\Phi_1 = \frac{Ax_{\text{DHAP}}}{B + Cx_{\text{DHAP}}} - \frac{Dx_{\text{DHAP}}^2}{E + Fx_{\text{DHAP}}^2} \quad (60)$$

where  $A = V_{\text{ex}}$ ,  $B = K_{\text{DHAP}} \left( 2 + \frac{x_{\text{P}_{\text{ext}}}}{K_{\text{P}_{\text{ext}}}} \right) \frac{x_{\text{P}_i}}{K_{\text{P}_i}}$ ,  $C = K_{\text{DHAP}} + 1 + \frac{x_{\text{P}_{\text{ext}}}}{K_{\text{P}_{\text{ext}}}}$ ,  $D = V_6 \frac{k_6 k_9}{k_7 k_8}$ ,  $E = 1 + K_{i62}^{-1} x_{\text{P}_i}$  and  $F = \frac{k_6 k_9}{k_7 k_8}$ . Using the positivity of the unknown we see that the equation  $\Phi_1 = 0$  is equivalent to the quadratic equation

$$(AF - CD)x_{\text{DHAP}}^2 - BDx_{\text{DHAP}} + AE = 0. \quad (61)$$

This equation has two positive solutions precisely when  $AF - CD > 0$  and  $AE < \frac{B^2 D^2}{4(AF - CD)}$ . Moreover in that case the derivative of  $\Phi_1$  is non-zero at each of those points. Parameters can be chosen such that these inequalities are satisfied. For instance, starting from arbitrary positive values of the parameters  $V_6$  can be reduced so as to ensure that the first inequality is satisfied. Then  $k_6$  can be increased to arrange that the second one is satisfied. Perturbing  $\zeta$  away from zero and applying the implicit function theorem we see that there exist two positive steady states of Model 3.4.1 for suitable choices of the parameters. This implies in turn the existence of two positive steady states for the Pettersson model. Summing up, we get the following result

**Theorem** There are choices of the parameters for which the Pettersson model has at least two positive steady states.

## 5 Steady states of the Poolman model

The equations for the steady state fluxes in the Poolman model are similar to those in the case of the Pettersson model. If we make use of the conservation law for the total amount of phosphate then the only difference is an additional summand  $v_{17}$  in the evolution equation for G1P. Note that this reaction rate belongs to a slow reaction. The explicit expression for this rate is  $v_{17} = \frac{V_{17} x_{\text{P}_i}}{x_{\text{P}_i} + K_{m17} \left( 1 + \frac{x_{\text{G1P}}}{K_{i17}} \right)}$  (cf. [15], equation (4.4)). The equations (54)-(58) in

[14] are replaced by

$$F_1 = v_{13} - v_1, \quad (62)$$

$$F_2 = v_6 - v_9 - v_{\text{st}} + v_{17}, \quad (63)$$

$$F_3 = v_6 + 2v_9 - v_{13} - v_{\text{st}} + v_{17}, \quad (64)$$

$$F_4 = 2v_1 + v_{\text{st}} - v_{\text{ex}} - 2v_9 - 3v_6 - v_{17}, \quad (65)$$

$$F_5 = v_{16} + v_{\text{PGA}} - 2v_1 - v_{13} - v_{\text{st}}. \quad (66)$$

Correspondingly equations (42)-(47) of [14] are replaced by

$$v_1 = v, \quad (67)$$

$$v_6 = \frac{v}{3} + v_{\text{st}} - v_{17}, \quad (68)$$

$$v_9 = \frac{v}{3}, \quad (69)$$

$$v_{13} = v, \quad (70)$$

$$v_{16} = 3v + v_{\text{st}} - v_{\text{PGA}}, \quad (71)$$

$$v = 3v_{\text{ex}} + 6v_{\text{st}} - 6v_{17}. \quad (72)$$

Let us freeze ATP and  $P_i$  in the reduced Poolman model and call the result Model 4.1.1. In the frozen model  $v_{17}$  is a function of  $x_{\text{G1P}}$  alone.

We now pass to a limit in a similar way to what was done for the Pettersson model, setting the reaction rates  $v_{\text{PGA}}$ ,  $v_{\text{PGA}}$  and  $v_{\text{st}}$  to zero. This time we allow  $v_{17}$  to remain non-zero. The calculations are simplified by setting  $K_{i17}^{-1}$  to zero, so that the expression for  $v_{17}$  reduces to a constant. Then equation (60) is replaced by

$$\Phi_1 = \frac{Ax_{\text{DHAP}}}{B + Cx_{\text{DHAP}}} - \frac{Dx_{\text{DHAP}}^2}{E + Fx_{\text{DHAP}}^2} - G \quad (73)$$

where  $G = \frac{3V_{17}x_{P_i}}{x_{P_i} + K_{m17}}$ . If we start from a choice of parameters which gives two positive steady states in the Pettersson model and perturb  $G$  from being zero to being positive and sufficiently small then the function  $\Phi_1$  has three non-degenerate zeroes. For a sufficiently small perturbation of this type does not destroy the positive zeroes present for  $G = 0$  and does not make them become degenerate. On the other hand it makes the value of  $\Phi_1$  at the origin negative and this leads to a new positive zero. This zero is a deformation of the zero which lies at the origin for  $G = 0$  and so it is non-degenerate for small parameter values. It follows by arguments similar to those in the last section that for the parameter values just considered the reduced Poolman model has three positive steady states.

Stoichiometric generators for the Poolman system have been studied in [16] and we now consider the relation of these to the construction just carried out. There are modes generalizing those for the Pettersson model by augmenting them by a zero entry for  $v_{17}$ . Strangely these do not seem to fit with Figure 2A in [16] where the reaction with flux  $v_7$  is also shown as being shut off. That figure appears to contradict equation (9) of [14] which says that in a steady



state  $v_6 = v_7 + v_{14}$ . We can now proceed as in the last section, with  $v_{17}$  being set to zero. We use the mode

$$[3 \ 6 \ 6 \ 3 \ 1 \ 1 \ 1 \ 1 \ 1 \ 1 \ 2 \ 3 \ 0 \ 0 \ 10 \ 0 \ 0 \ 1 \ 0 \ 0]. \quad (74)$$

That takes us to the same subnetwork as before and the analogous arguments show that there are parameter values for which the reduced Poolman model has two positive steady states. As has been shown above the possibility of having  $v_{17}$  non-zero to get results for the reduced Poolman model which go beyond those obtained for the Pettersson model.

To obtain these modes it is necessary to be careful about the difference between  $N$  and  $\bar{N}$ . This time we will make a different choice of which reactions are considered to be in the forward direction. This results in the reaction rates  $v_{14}$  and  $v_{15}$  being replaced by their negatives. This is related to the fact that in this mode material is flowing from starch to the sugars in the cycle. Consider the mode

$$[3 \ 6 \ 6 \ 4 \ 0 \ 0 \ 1 \ 1 \ 1 \ 1 \ 1 \ 2 \ 3 \ 1 \ 1 \ 9 \ 0 \ 0 \ 1 \ 0 \ 1]. \quad (75)$$

In this case we get a subnetwork with an inflow from starch but no outflow to starch.

Note finally that in this paper we have not obtained any results on multiple stationary solutions for the Pettersson model itself or for the hybrid model. In order to do this it would be necessary to obtain information on the transverse eigenvalues, showing that they are all different from zero, at least for some values of parameters for which multiple steady states exist for the reduced models.

## 6 Outlook

In this paper we have been concerned with a variety of mathematical models for one biological system, the Calvin cycle. The approach has been to understand as much as possible about the relations between the different models and to obtain as much information as possible about the qualitative behaviour of the solutions of the equations defined by these models. The scope was restricted to results obtained by purely analytical and rigorous methods without any appeal to numerics or reliance on heuristics. These results are piecemeal and should be complemented by a better conceptual understanding of the key mechanisms determining the behaviour. To do this it makes sense to look at models which are as simple as possible.

The approach of studying the simplest possible model has been pursued by Hahn [8]. His three-variable model includes the important phenomenon of photorespiration which is not included in the models discussed up to now in this paper. Because this seems to the author to be an important direction for future developments the work of [8] will now be discussed briefly. The unknowns are  $x_{\text{RuBP}}$ ,  $x_{\text{PGA}}$  and  $x_{\text{TP}}$ . TP stands for 'triose phosphate' and compared to the five-variable models it is obtained by lumping together  $x_{\text{DPGA}}$  and  $x_{\text{GAP}}$ .  $x_{\text{Ru5P}}$  has been considered as an intermediate species and discarded. Let us

ignore photorespiration for the moment ( $k_2 = 0$  in the notation of [8]). We also ignore the reaction called dark respiration ( $k_5 = 0$ ).

The reaction from RuBP to PGA is as in Model 2.1.1 and there is a reaction taking PGA to TP replacing that from PGA to DPGA in Model 2.1.1. There is a sink reaction starting at TP. There is a reaction from TP to RuBP replacing that from GAP to Ru5P. The evolution equations are

$$\frac{dx_{\text{RuBP}}}{dt} = -k_1 x_{\text{RuBP}} + 3k_4 x_{\text{TP}}^5, \quad (76)$$

$$\frac{dx_{\text{PGA}}}{dt} = 2k_1 x_{\text{RuBP}} - k_3 x_{\text{PGA}}, \quad (77)$$

$$\frac{dx_{\text{TP}}}{dt} = k_3 x_{\text{PGA}} - 5k_4 x_{\text{TP}}^5 - k_6 x_{\text{TP}}. \quad (78)$$

It is emphasized by Hahn that a key property which is expected from a model is that it should have a stable positive steady state. He states that  $k_1$  can reasonably be estimated from experimental data but that  $k_3$ ,  $k_4$  and  $k_6$  cannot. Thus he adopts the following strategy. It is assumed that a stable steady state exists in the model and the concentrations of the substances involved which can be measured under suitable circumstances are assumed to be the values in the steady state. Assuming in this way certain values for the coordinates of the steady state the three remaining reaction constants can be calculated. Having obtained these values we can then ask if for those parameters there are other positive steady states.

The system above has exactly one positive steady state for any values of the parameters. It satisfies  $x_{\text{TP}} = \left(\frac{k_1 x_{\text{RuBP}}}{3k_4}\right)^{\frac{1}{5}}$ ,  $x_{\text{PGA}} = \frac{2k_1 x_{\text{RuBP}}}{k_3}$  and

$$x_{\text{RuBP}} = \left[ \left( \frac{k_1}{3k_6} \right)^5 \frac{3k_4}{k_1} \right]^{\frac{1}{4}}. \quad (79)$$

Note that in contrast to Model 2.1.1 this model always has a positive steady state for any choice of the parameters. The difference has to do with the fact that in the Hahn model there is no sink for  $x_{\text{PGA}}$ . It is shown in [8] that this steady state is unstable.

In the case with photorespiration it is shown in [8] how to reduce the problem of finding steady states to that of solving a ninth degree equation for one of the concentrations. Numerically it was found that for the biologically motivated values of the parameters this model has two steady states. Moreover one of these is stable and the other unstable. This is similar to the results which have proved for other models discussed in the previous sections. In the model of [8] this situation is only possible in the presence of photorespiration. However the other models suggest that in this respect Hahn's three-variable model is not representative of what happens in more detailed models. It is desirable to obtain more analytical results on the Hahn models and their relations to other more detailed models of the Calvin cycle. Note that in [7] Hahn had also previously studied some larger models which have rise to the models of [8] by a process of simplification.

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